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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,235,119**, on April 17, 1998, by **KENNETH CURRY AND JOHN CHEN**, for "Novel Glutamate Receptor Chemicals"

PRIORITY DOCUMENT

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S. Gregoire

Agent certificateur / Certifying Officer

April 27, 1999

Date

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NOVEL GLUTAMATE RECEPTOR CHEMICALS

ABSTRACT

These compounds that demonstrate either activating or inhibiting activity at various glutamate receptors (GluRs) (i.e., ionotropic or metabotropic) have therapeutic potential for the treatment of neurological disorders through interactions at the GluR class of brain receptors. These compounds have application as new drugs to treat both acute and chronic neurological disorders, such as stroke and head injuries; epilepsy; movement disorders associated with Parkinson's Disease and Huntington's chorea; pain; anxiety; AIDS dementia; Alzheimer's disease. Since the GlurRs can influence levels of alertness, attention and cognition; protect nerve cells from excitotoxic damage resulting from ischemia, hypoglycemia and anoxia; modulate the level of neuronal excitation; influence central mechanisms involved in controlling movement; reduce sensitivity to pain; reduce levels of anxiety, these compounds can also be used to influence these situations and find use in learning and memory.

FIELD OF THE INVENTION

This invention pertains to chemical compounds for use at ionotropic and metabotropic glutamate receptors.

BACKGROUND OF THE INVENTION

The billions of cells (neurons) in the brain and spinal cord, the Central Nervous System (CNS) of mammals transmit information from cell to cell by releasing chemicals known as neurotransmitters through their cell membranes. Subsequent to their release, the neurotransmitters evoke a response from their target cell by interacting with different classes of receptors on the target cell's membrane. The evoked response can be inhibitory, excitatory or modulatory, and occurs as a result of the controlled movement of ions such as sodium, potassium, chloride and calcium across the cell membrane.

Errors in neurotransmission or the uncontrolled movements of ions across the cell membrane can lead to neuropathological conditions and *vice versa*. Sodium and calcium overload of neurons is thought to be a critical factor in the initiation of the pathological conditions leading to cell death following the cerebral ischemia (anoxia) that occurs during strokes or traumatic head injuries, for example. Ischemia of neurons leads to depolarization, potassium loss, sodium uptake with associated cellular swelling, calcium uptake, calcium accumulation in mitochondria with associated damage, liberation of neurotransmitters and activation of calcium-dependent enzymes.

The need for new pharmaceuticals to treat acute neurological disorders is critical. For example, in Canada alone it is estimated that approximately 50,000 Canadians suffer from strokes each year and of these 15,000 will die. The remainder, like the 15,000 who suffer from some form of head injury each year, are left with varying degrees of permanent disability that inflicts extraordinary hardships on the individual and costs the country a staggering amount in health care costs.

The human and financial toll that results from acute neurological situations is compounded by chronic neurological disease states such as Parkinson"s and Alzheimer's Diseases and Huntington's chorea. These devastating disorders become more prevalent as individuals age and thus, with the trend continuing towards greater longevity worldwide, these disorders will continue to increase in numbers. This in turn will increase the already staggering amounts of medical expenditures that are allocated to dealing with these diseases. The unfortunate consequence of the increases in both acute and chronic human neurological disorders (and the lack of existing remedies) is that an immense market currently exists worldwide for drug-based treatments.

The current pharmaceutical options for treating neurological disorders also tend to be very general and non-specific in their actions in that, although they may reduce the clinical symptoms associated with a specific neurological disorder, they may also negatively impact normal function of the Central Nervous System of patients. Thus new cellular targets and drugs that are more specific in their actions need to be identified and developed.

The acidic amino acid L-glutamic acid (L-Glu) is recognized as the major excitatory neurotransmitter in the CNS. Research has revealed that L-Glu has both "fast" (ionotropic) neurotransmitter actions, and slower (metabotropic), modulatory effects, evoked through eight distinct receptor subclasses.

Ionotropic glutamate receptors contain integral, cation-specific ion channels, whereas the metabotropic receptors are coupled to G proteins and modulat the production of intracellular messengers. The ionotropic receptors can be subdivided into N-methyl-D-aspartate (NMDA) receptors and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-kainate receptors according to their selective agonists.

Much of the current research in this area is focused on developing chemicals that will interact at a new class of brain receptors. These metabotropic glutamate receptors (mGluRs), or which there are eight distinct types, were only identified within the last ten years. The receptor subtypes are differentiated by their pharmacological profiles as well as by the intracellular second messenger effects that they evoke. For example, L-Glu's interaction at the mGluR1 and mGluR5 subclass of receptor activates phospholipase C and a subsequent inositol phosphate cascade via a

G-protein.

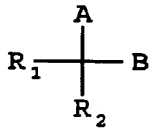
Research directed towards mGluRs is beginning to show that mGluRs may be implicated in a number of normal as well as pathological mechanisms in the brain and spinal cord. For example, activation of these receptors on neurons can: influence levels of alertness, attention and cognition; protect nerve cells from excitotoxic damage resulting from ischemia, hypoglycemia and anoxia; modulate the level of neuronal excitation; influence central mechanisms involved in controlling movement; reduce sensitivity to pain; reduce levels of anxiety.

The consequence of designing chemicals which interact at these receptor sites is that it will be possible to develop therapeutics with the potential to target areas such as: learning and memory; stroke and head injuries; epilepsy; movement disorders associated with Parkinson's Disease and Huntington's chorea; pain; anxiety; AIDS dementia; Alzheimer's disease.

Thus, a need remains for chemical compounds that demonstrate specific binding characteristics towards mGluRs, in addition to the ongoing research into ligands for ionotropic glutamate receptors.

SUMMARY OF THE INVENTION

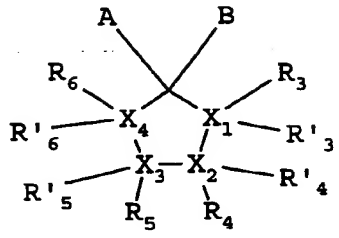
It is an object of this invention to provide novel compounds that demonstrate activity at the various glutamate receptors. In particular, a compound of formula (I) and stereoisomers thereof:



(I),

wherein:

(i) R_1 and R_2 together form a five membered ring thereby providing a compound of formula (II):



(II),

wherein:

X_1, X_2, X_3, or X_4, can be C and up to **three** of X_1, X_2, X_3, and X_4, can be selected from the group consisting of S, O and N;

if present, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, and R'_6 can be -H, aliphatic, aromatic, carboxycyclic and heterocyclic, or Cl, Br, F, NO_2, or, SY or NY, wherein Y can be -H, aliphatic, or aromatic;

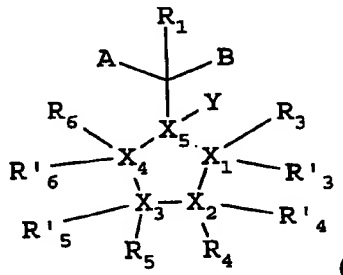
either R_3 or R_4 can be A;

R_3 and R'_3 can form a spiropropane-A group;

R_4 and R'_4 can form a spiropropane-A group;

either R_4 and R_5 together, or R_5 and R_6 together can form an aromatic ring or aliphatic ring; and at least two acidic groups and one basic group are present, or

(ii) R_2 is a five membered ring thereby providing a compound of formula (III):



(III)

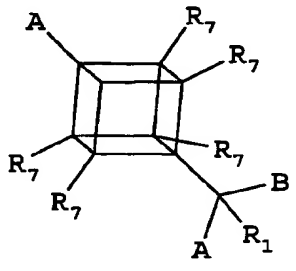
wherein:

X_1, X_2, X_3, or X_4, can be C and up to **three** of X_1, X_2, X_3, and X_4, can be selected from the group

consisting of S, O and N;

R_1 can be -H, aliphatic, or aromatic;
if present, Y, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, and R'_6 can be -H, aliphatic, or aromatic;
either R_3 or R_4 can be A;
R_3 and R'_3 can form a spiropropane-A group;
R_4 and R'_4 can form a spiropropane-A group;
either R_4 and R_5 together or R_5 and R_6 together can form an aromatic ring or aliphatic ring; and
at least two acidic groups and one basic group are present, or

(iii) R_2 is a cubane ring thereby providing a compound of formula (IV)



(IV),

wherein:

R_1 can be -H, alipatic or aromatic;
R_7 can be the same or different and -H, aliphatic, aromatic, carbocyclic, heterocyclic, Cl, Br, F, NO_2, or OR, or two or more adjacent R_7 can form a aromatic, aliphatic. carbocyclic, or heterocyclic ring,

wherein in formula (II), (III) and (IV), at least two acidic groups and one basic group are present wherein
A can be an acidic group selected from the group consisting of carboxy, phosphono, phosphino, sulfono, sulfino, borono, tetrazol, isoxazol, -CH_2-carboxy, -CH_2-phosphono, -CH_2-phosphino, -CH_2-sulfono, -CH_2-sulfino, -CH_2-borono, -CH_2-tetrazol, and -CH_2-isoxazol;

B can be a basic group selected from the group consisting of 1° amino, 2° amino, 3° amino, quaternary ammonium salts, aliphatic 1° amino, aliphatic 2° amino, aliphatic 3° amino, aliphatic quaternary ammonium salts, aromatic 1° amino, aromatic 2° amino, aromatic 3° amino, aromatic quaternary ammonium salts, imidazol, guanidino, boronoamino, allyl;

and pharmaceutrically acceptable salts thereof.

This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for exerting inhibitory or activating activity at GluRs, and may be advantageously used as agents to treat both acute and chronic neurological disorders, such as stroke and head injuries; epilepsy; movement disorders associated with Parkinson's Disease and Huntington's chorea; pain; anxiety; AIDS dementia; Alzheimer's disease. Since the GluRs can influence levels of alertness, attention and cognition; protect nerve cells from excitotoxic damage resulting from ischemia, hypoglycemia and anoxia; modulate the level of neuronal excitation; influence central mechanisms involved in controlling movement; reduce sensitivity to pain; reduce levels of anxiety, these compounds can also be used to influence these situations and find use in learning and memory.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows agonist activities of (s)-Glu and of homo-ACPD's at CHO cells expressing (A) $mGlu_{1\alpha}$, (B) $mGlu_2$ and (C) $mGlu_{4a}$. In agonist assays $mGlu_{1\alpha}$-expressing cells were incubated with ligand at a concentration of 1 mM for 20 min. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 20 min in the presence of 10 μM (S)-Glu. Total IP formation was determined by an ion-exchange assay and the fold increase in IP level calculated compared to control cells (incubated in buffer only). In agonist assays $mGlu_2$ and $mGlu_{4a}$-expressing cells were incubated with ligands (1 mM) for 10 min in the presence of 10 μM forskolin. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 10 min in the presence of 20 μM ($mGlu_2$) or 50 μM ($mGlu_{4a}$) (S)-Glu and 10 μM forskolin. Cyclic AMP levels were measured by a RIA assay and expressed as percent of cyclic AMP level in control cells (incubated in buffer only). Data are the means (±SD) of representative experiments performed in triplicate.

Figure 2 shows (A) Dose-response curves of (S)-Glu in the absence (●) or presence (■) of 1 mM (1RS,2RS)-homo-ACPD at $mGlu_2$-expressing cells. (B) Dose response curves of (1S,3R)-homo-ACPD (●) and (1R,3R)-homo-ACPD (■) at $mGlu_2$-expressing cells. For further details, see

legend for Figure 2.

Table 1 shows a pharmacologic description of certain compounds of this invention at representative mGlu receptor subtypes expressed in Chinese hamster ovary (CHO) cells.

DETAILED DESCRIPTION OF THE INVENTION

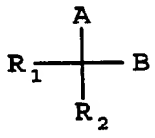
The mGluR compounds of this invention are characterized by specific structural and physiochemical features.

This invention comprises a family of compounds, whose common theme concerns the activity and predictability of rigid and restricted analogues of the amino acid, L-glutamic acid (L-Glu). In general, a degree of rigidity is needed within these structures and this is coupled to the preferred L- or S- configuration at the α amino acid carbon.

Substitution at the α amino carbon decreases agonist activity at ionotropic L-glu receptors and subsequently increases specificity (but not necessarily potency) at mGluRs. Moreover, in addition to the above requirements, the agonists are relatively folded while antagonists are often more extended and unsaturated. In addition, activity at the mGluR2 and mGluR3 receptors is achieved with more extended molecules and a combination of unsaturation and a hyper-extended L-Glu equivalent leads to antagonism at these receptors.

The core to this family of compounds is cyclopentyl- and cyclopropyl-bound L-Glu.

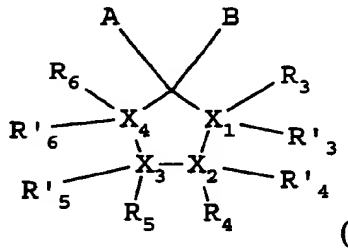
Compounds of this invention that meet the stereochemistry requirements of GluR agonists or antagonists are compounds of formula (I) and stereoisomers thereof:



(I),

wherein:

(i) R_1 and R_2 together form a five membered ring thereby providing a compound of formula (II):



(II),

wherein:

X_1, X_2, X_3, or X_4, can be C and up to **three** of X_1, X_2, X_3, and X_4, can be selected from the group consisting of S, O and N;

if present, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, and R'_6 can be -H, aliphatic, aromatic, carboxycyclic and heterocyclic, or Cl, Br, F, NO_2, and OR;

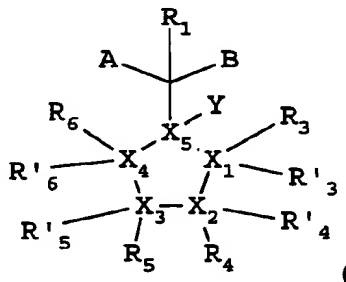
either R_3 or R_4 can be **A**;

R_3 and R'_3 can form a spiropropane-**A** group;

R_4 and R'_4 can form a spiropropane-**A** group;

either R_4 and R_5 together, or R_5 and R_6 together can form an aromatic ring or aliphatic ring; and at least two acidic groups and one basic group are present, or

(ii) R_2 is a five membered ring thereby providing a compound of formula (III):



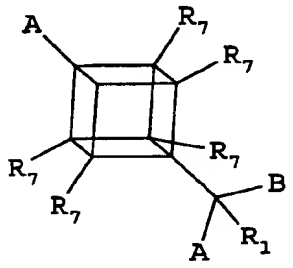
(III)

wherein:

X_1, X_2, X_3, or X_4, can be C and up to **three** of X_1, X_2, X_3, and X_4, can be selected from the group consisting of S, O and N;

R_1 can be -H, aliphatic, or aromatic;
if present, Y, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, and R'_6 can be -H, aliphatic, or aromatic;
either R_3 or R_4 can be **A**;
R_3 and R'_3 can form a spiropropane-**A** group;
R_4 and R'_4 can form a spiropropane-**A** group;
either R_4 and R_5 together or R_5 and R_6 together can form an aromatic ring or aliphatic ring; and
at least two acidic groups and one basic group are present, or

(iii) R_2 is a cubane ring thereby providing a compound of formula (IV)



(IV),

wherein:

R_1 can be -H, alipatic or aromatic;
R_7 can be the same or different and -H, aliphatic, aromatic, carbocyclic, heterocyclic, Cl, Br, F, NO_2, or OR, or two or more adjacent R_7 can form a aromatic, aliphatic. carbocyclic, or heterocyclic ring,

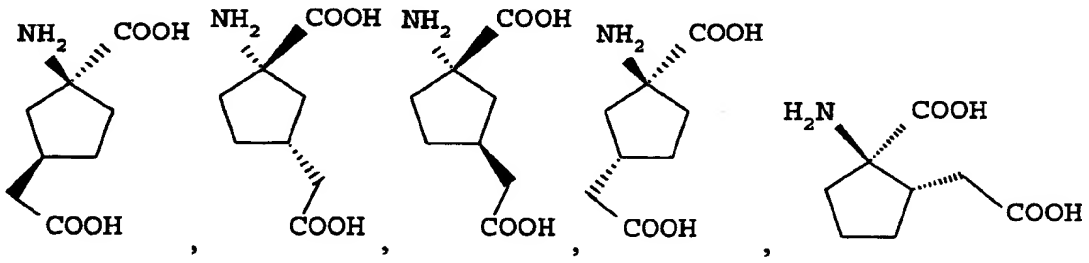
wherein in formula (II), (III) and (IV), at least two acidic groups and one basic group are present wherein
A can be an acidic group selected from the group consisting of carboxy, phosphono, phosphino,

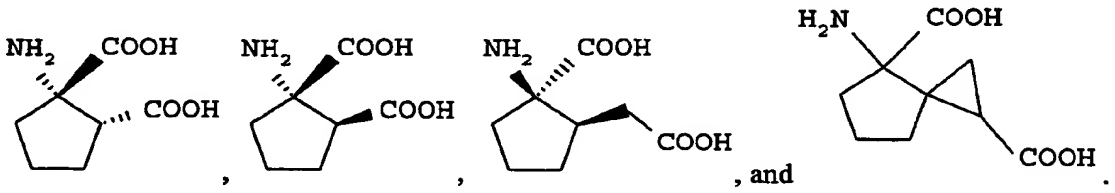
sulfono, sulfino, borono, tetrazol, isoxazol, -CH_2-carboxy, -CH_2-phosphono, -CH_2-phosphino, -CH_2-sulfono, -CH_2-sulfino, -CH_2-borono, -CH_2-tetrazol, and -CH_2-isoxazol;

B can be a basic group selected from the group consisting of 1° amino, 2° amino, 3° amino, quaternary ammonium salts, aliphatic 1° amino, aliphatic 2° amino, aliphatic 3° amino, aliphatic quaternary ammonium salts, aromatic 1° amino, aromatic 2° amino, aromatic 3° amino, aromatic quaternary ammonium salts, imidazol, guanidino, boronoamino, allyl;

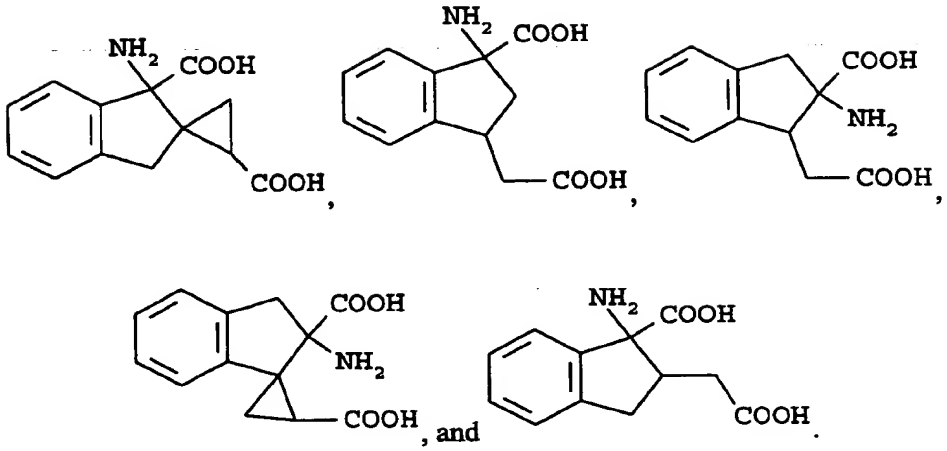
and pharmaceutically acceptable salts thereof.

In particular compounds wherein the compound of formula (I) is selected from the group consisting of:

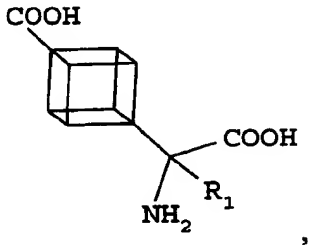




or wherein the compound of formula (I) is selected from the group consisting of:



or wherein the compound of formula (I) is:



wherein:

R_1 can be -H or -Me.

The present invention also provides pharmaceutical compositions containing a compounds as disclosed in the claims in combination with one or more pharmaceutically acceptable, inert or physiologically active, diluents or adjuvants. The compounds of the invention can be freeze dried and, if desired, combined with other pharmaceutically acceptable excipients to prepare formulations for administration. These compositions may be presented in any form appropriate

for the administration route envisaged. The parenteral and the intravenous route are the preferential routes for administration.

Compounds of the general Formula I may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition, there is provided a pharmaceutical formulation comprising a compound of general Formula I and a pharmaceutically acceptable carrier. One or more compounds of general Formula I may be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients. The pharmaceutical compositions containing compounds of general Formula I may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared according to any known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate: granulating and disintegrating agents for example, corn starch, or alginic acid: binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia: dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or *n*-propyl *p*-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oils phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monoleate, and condensation products of the said partial esters

with ethylene oxide, for example polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulation according to known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compound(s) of the general Formula I may be administered, together or separately, in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Compound(s) of general Formula I may be administered, together or separately, parenterally in sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetics, preservatives and buffering agents can be dissolved in the vehicle.

The mode, dosage and schedule of administration of taxol in human cancer patients has been studied extensively (see Ann. Int. Med. 111:273 1989). For the compounds of this invention, the dose to be administered, whether a single dose, multiple dose, or a daily dose, will vary with the particular compound being used. Factors to consider when deciding upon a dose regimen include potency of the compound, route of administration, size of the recipient and the nature of the patient's condition.

The dosage to be administered is not subject to defined limits, but in will usually be an effective amount. It will usually be the equivalent, on a molar basis of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects.

EXAMPLES

Example I: Pharmacological Demonstration of Certain Compounds at Representative mGlu Receptor Subtypes Expressed in Chinese Hamster Ovary (CHO) Cells.

The Chinese hamster ovary cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$ receptors have been described previously (Amarori and Nakanishi, *Neuron* 8, 757-765, 1992; Tanabe *et al.*, *Neuron* 8, 169-179, 1992, and *J. Neurochem.* 63, 2038-2047, 1993). They were maintained at 37 °C in a humidified 5% CO_2 incubator in Dubecco's Modified Eagle Medium (DMEM) containing a reduced concentration of (S)-glutamine (2mM) and were supplemented with 1% proline, penicillin (100 U/ml), streptomycin (100 mg/ml) and 10% dialyzed fetal calf serum (all GIBCO, Paisley). Two days before assay 1.8×10^6 cells were divided into the wells of 24 well plates.

PI hydrolysis was measured as described previously (Hayashi *et al.*, *Nature* 366, 687-690,1992, and *J. Neurosci.* 14, 3370-3377, 1994). Briefly, the cells were labeled with [^{3}H]inositol (2μ Ci/ml) 24 h prior to the assay. For agonist assays, the cells were incubated with ligand dissolved in phosphate-buffered saline (PBS)-LiCl for 20 min, and agonist activity was determined by measurement of the level of ^{3}H-labeled mono-, bis- and tris-inositol phosphates by ion-exchange chromatography. For antagonist assays, the cells were preincubated with the ligand dissolved in PBS-LiCl for 20 min prior to incubation with ligand and 10 μM (S)-Glu for 20 min. The antagonist activity was then determined as the inhibitory effect of the (S)-Glu mediated response. The assay of cyclic AMP formation was performed as described previously (Hayashi *et al.*, 1992, 1994). Briefly, the cells were incubated for 10 min in PBS containing the ligand and 10 μM forskolin and 1 mM 3-isobutyl-1-methylxanthine (IBMX) (both Sigma, St. Louis, MO, USA). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic

AMP formation. For antagonist assay, the cells were preincubated with ligand dissolved in PBS containing 1 mM IBMX for 20 min prior to a 10 min incubation in PBS containing the ligand, 20 μM($mGlu_2$) or 50 μM ($mGlu_{4a}$) (S)-Glu, 10 μM forskolin and 1 mM IBMX.

All experiments were performed in triplicate and the results are given as mean ± S.E.M. of at least three independent experiments. Antagonist potency was calculated from the Gaddum equation KB = [B]/(DR - 1) (Lazareno and Birdsall, *Trends Pharmacol. Sci.* 14, 237-239,1993), were the dose-ratio (DR) is the ratio of the EC_{50} values of (S)-Glu in the presence and in the absence of a fixed antagonist concentration, [B].

The structures of the homo-ACPD's tested in this study are described in the legend of Figure 1 of the Brief Description of Drawings. These ligands were initially tested in 1 mM concentration at CHO cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$ receptors representing group I, II and III, respectively. As seen in Figure 2 none of the homo-ACPD's had any effect at $mGlu_{1\alpha}$ (panel A) or $mGlu_{4a}$ (panel C) when added alone or in combination with a submaximal concentration of (S)-Glu indicating that these ligands are inactive as agonists and antagonists at these receptors. (1 RS, 2 RS)-Homo0ACPD had no activity at $mGlu_2$ when added alone, but could partially antagonize the (S)-Glu induced response indicating antgonism. Consistent with this result, 1 mM (1 RS, 2RS)-homo-ACPD caused a right-ward shift of the dose-response curve for (S)-Glu (Fig 3A). Using the Gaddum equation a KB value of 391 μM was determined for (1 RS, 2RS)-homo-ACPD (Table 1). (1 S, 3R)-homo-ACPD and (1 R, 3R)-homo-ACPD both displayed significant agonist activity when tested in 1mM (Fig. 2B). When examining the dose-response curves (Fig 3B) for (1 S, 3R)-homo-ACPD and (1R,3R)-homo-ACPD EC50 values of 122 μM and 105 μM, respectively, were obtained (Table 1). As seen in Fig. 3 and Table 1 both compounds were partial agonists with intrinsic activities of 79% and 47%, respectively, compared to the maximal response of (S)-Glu. When tested at 1 mM concentration (1S, 3S)-homo-ACPD was both able to decrease the cyclic AMP level by 27 ± 4% compared to the maximal response of (S)-Glu and to antagonize partially the (S)-Glu induced responses. This indicates that (1S, 3S)-homo-ACPD is a partial agonist, however, the response was too weak to measure a reliable EC_{50} value. Finally, (1R, 3S)-homo-ACPD was found to be inactive at the mGlu2 receptor (Fig. 2).

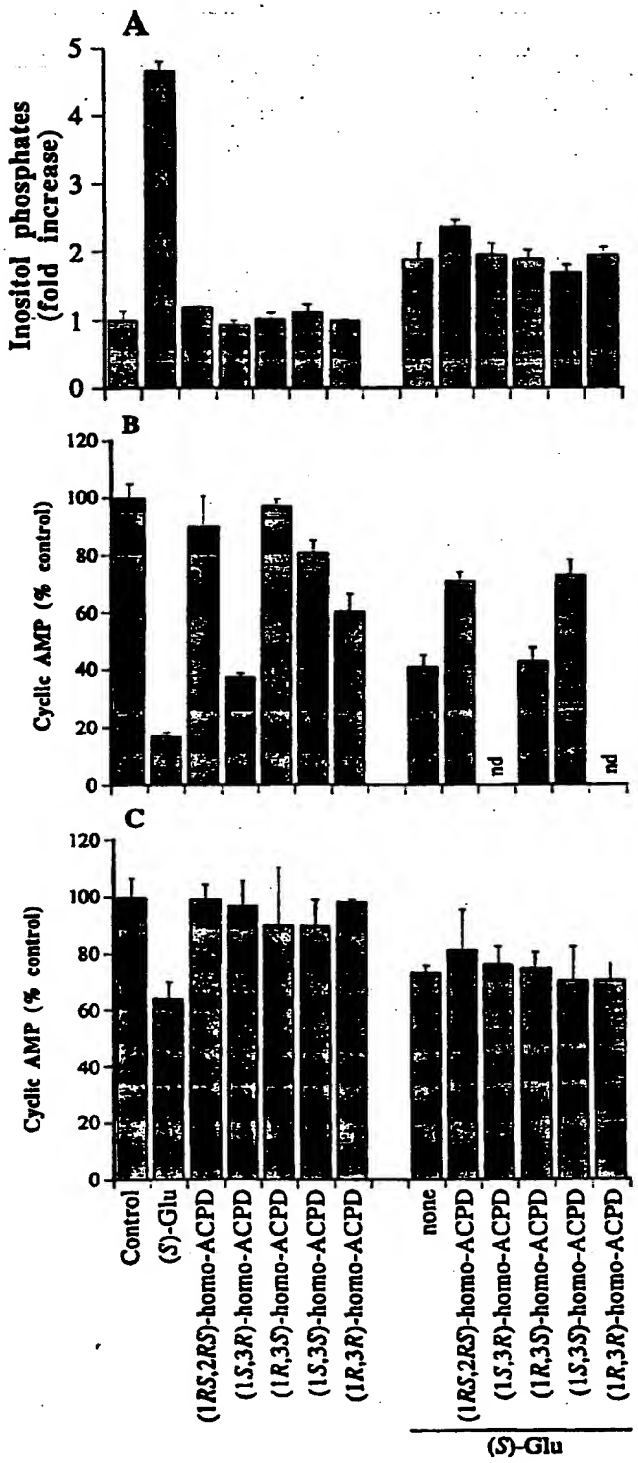
No effects of the five homo-ACPD's was observed at 1 mM concentration when tested as

agonists or antagonists at ionotropic Glu receptors using the cortical wedge preparation of rat neocortex.

Table 1
Pharmacological activity of ligands at the mGlu receptor subtypes expressed in CHO cells

	EC_{50} (μM) [a] (% of maximal (*S*)-glu response)		
	$mGlu_{1\alpha}$	$mGlu_2$	$mGlu_{4a}$
(*S*)-glu	19 ± 2 (100%)	7.7 ± 0.7 (100%)	21 ± 2 (100%)
(*S*)-2-aminoadipic acid [b]	> 1000	35 ± 1	> 3000
(1*SR*,3*RS*)-ACPD	121 ± 6 (83 ± 11%)	11 ± 1 (105 ± 1%)	~ 1000 [c]
(1*RS*,2*RS*)-homo-ACPD	> 1000	391 ± 89 [d]	> 1000
(1*S*,3*R*)-homo-ACPD	> 1000	122 ± 49 (79 ± 4%)	> 1000
(1*R*,3*S*)-homo-ACPD	> 1000	> 1000	> 1000
(1*S*,3*S*)-homo-ACPD	> 1000	p.a. (27 ± 4%)	> 1000
(1*R*,3*R*)-homo-ACPD	> 1000	105 ± 36 (47 ± 9%)	> 1000

[a] Mean ± standard error of mean of at least three independent experiments.
[b] From Bräuner-Osborne et al. (1996).
[c] Estimated from partial dose–response curves.
[d] K_B value calculated using the Gaddum equation. p.a., partial agonist, response too weak to measure a reliable EC_{50} value.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1
2
3
4
5
B
Cyclic AMP (% control)
0
20
40
60
80
100
120
nd
nd
C
Cyclic AMP (% control)
0
20
40
60
80
100
120
Control
(S)-Glu
(1RS,2RS)-homo-ACPD
(1S,3R)-homo-ACPD
(1R,3S)-homo-ACPD
(1S,3S)-homo-ACPD
(1R,3R)-homo-ACPD
none
(1RS,2RS)-homo-ACPD
(1S,3R)-homo-ACPD
(1R,3S)-homo-ACPD
(1S,3S)-homo-ACPD
(1R,3R)-homo-ACPD
(S)-Glu

Figure 1

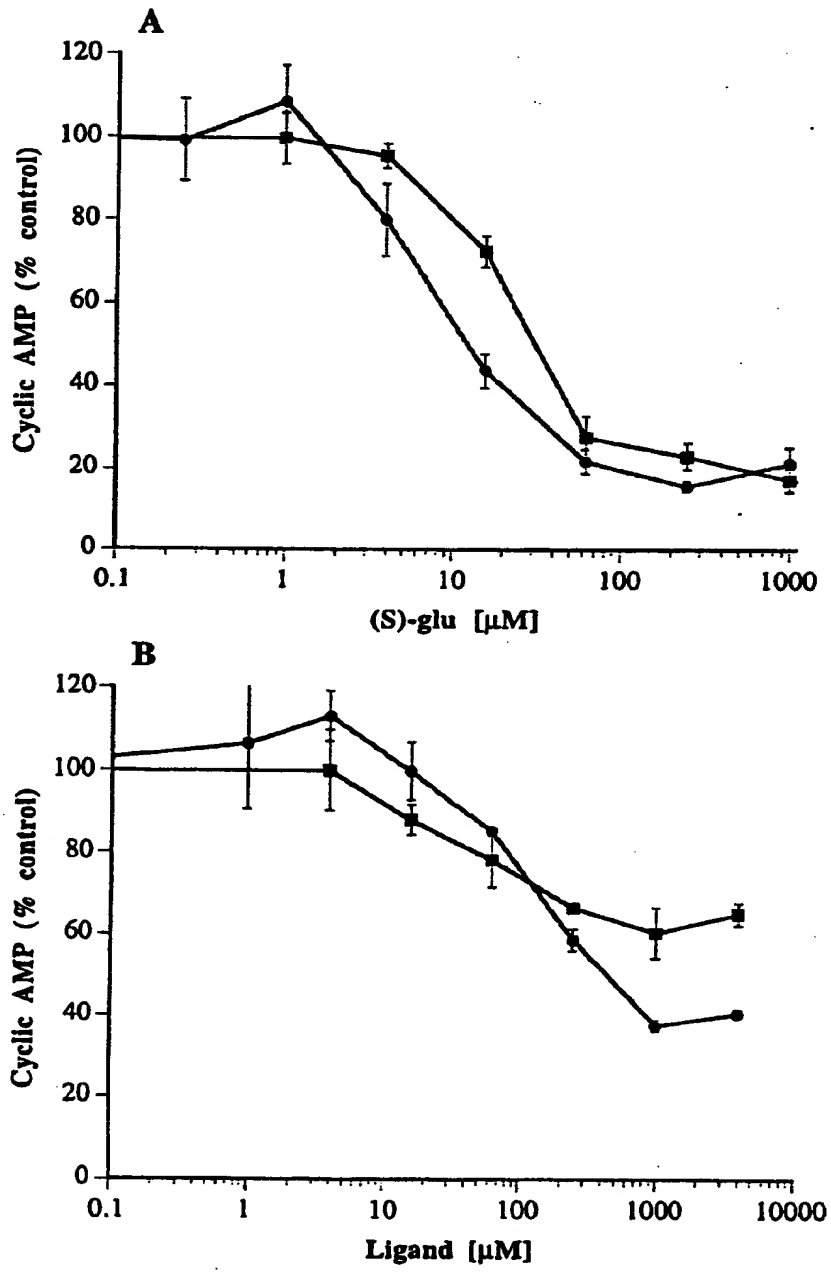
A
120
100
80
60
40
20
0
Cyclic AMP (% control)
0.1
1
10
100
1000
(S)-glu [µM]
B
120
100
80
60
40
20
0
Cyclic AMP (% control)
0.1
1
10
100
1000
10000
Ligand [µM]

Figure 2